JMGCB
ISSN:3032-1085
https://doi.org/10.61796/jmgcb.v1i5.511

Isolation and diagnosis of Staphylococcus aureus from wounds and detection of some virulence factors

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Received: March 22, 2024; Accepted: Apr 29, 2024; Published: May 28, 2024;

Abstract: The current study aimed to isolate and diagnose S. aureus, test its antibiotic susceptibility, and detect some virulence factors. 150 Clinical samples were collected from Kirkuk Hospital in Kirkuk city for the period from December 2023 to March 2024 from patients who were admitted and hospitalized after consulting the specialist doctor and referring him to the laboratory. Colonies of S. aureus were diagnosed based on the culturing characteristics of the colonies growing on mannitol salt agar medium and blood agar media and incubated at 37 0C for 24 hrs. The results showed that 47 (31.3%) of the samples showed bacterial growth. On the other hand, the results showed that S. aureus was highly resistant to Benzylpenicillin (81.8%), while it was very sensitive to both Nitrofurantion and Gentamicin (90.9%). A DNase test was conducted for clinical isolates of S. aureus, which were isolated from burns and wounds, and the results were 100% in terms of the susceptibility of S. aureus isolates to DNase results. The ability of 47 isolates of S. aureus to produce hemolysin was tested by culturing the bacteria on blood agar medium. The ratios of S. aureus isolates to haemolysin production varied, as evidenced by the data, with 39 (82.98%) isolates exhibiting complete hemolysis. It is concluded from the current study that S. aureus that was isolated from wounds was highly resistant to antibiotics and had dangerous harmful factors that increased the bacterial level in infections..

Keywords: S. aureus; DNase; Hemolysin; Antibiotics.

Introduction

Human skin protects the underlying tissues, bones, and organs from infection by acting as an effective barrier [1-2]. A wound is defined as a rupture in the skin's or tissues' structural integrity that reduces the skin's ability to defend itself [3]. Seventy to eighty percent of deaths following burn injuries are related to wound infection, one of the leading causes of mortality and morbidity in surgical patients [4]. Pyoderma moderate to severe necrotizing infections are examples of skin infections. A wide range of microorganisms, such as fungi, bacteria, and parasites, can infect skin. Bacteria are the most common cause of skin infections. The most frequent gram-positive bacteria that cause skin infections are Staphylococcus aureus and hemolytic streptococcus [5]. Worldwide, Staphylococcus aureus is a significant bacterial pathogen that can cause hospital-acquired infections as well as infections in the community. Methicillin-resistant S. aureus (MRSA) in particular has become a worldwide health hazard because of its resistance to beta lactam and other antibiotic groups [6-7]. Numerous illnesses in both people and animals are caused by the bacteria Staphylococcus. Gram-positive aerobic facultative bacteria, S. aureus, is non-motile and usually found in pairs [8]. It is a serious infection that colonizes and infects both healthy individuals with normal immune systems and hospital patients with weakened immune systems. The human body's nasopharynx and skin are home

to these naturally occurring microorganisms. S. aureus on the surface of the skin may lead to an infection if there is a rupture in the skin due to an accident or surgery, or if the person's immune system is compromised [9-10]. Primarily found on mucosal surfaces, S. aureus is frequently implicated in numerous nosocomial infections [11]. In order to investigate S. aureus's drug susceptibility and identify potential virulence factors, the current study set out to isolate and diagnose the bacteria.

Methods

150 Clinical samples were collected from Kirkuk Hospital in Kirkuk city for the period from December 2023 to March 2024 from patients who were admitted and hospitalized after consulting the specialist doctor and referring him to the laboratory.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics

Based on the culturing features of the S. aureus colonies developing on blood agar and mannitol salt agar media, the colonies were diagnosed and incubated for 24 hours at 37 0C.

Direct examination

By using a microscope to examine the morphological characteristics of germ cells—specifically, how they contacted the gram stain, which indicates the kind of interaction as well as the shape and arrangement of the germ cells—bacterial colonies were found.

Biochemical reaction and motility test

Numerous biochemical tests, such as the H2S production, TSI reaction, manitol fermentation, methyl red, citrate, urease, voges-proskauer, catalase, oxidase, and indole assays, were carried out in order to identify and diagnose bacteria.

Antibiotic susceptibility test (AST)

In accordance with clinical laboratory standards institute (CLSI) guidelines, the AST for all isolates was carried out using the Kirby-Bauer disc diffusion method using Muller Hinton (MH) agar (CLSI, 2020) [12-13]. The antibiotics discs (Bioanalyse(USA)) used in this study were Benzylpenicillin (10 µg) , Oxaccillin (1µg) Gentamicin (10mg) Tobramycin (30µg), Levofloxacin (5µg), Moxifloxacin (30µg), Erythromycin (15µg), Clindamycin (10µg), Vancomycin (30 µg), Nitrofurantion (30mg), Trimethoprim (5mg). Multidrug-resistant (MDR) organisms were defined as those that exhibited resistance to several antimicrobial agent types, classes, or subclasses [14].

Virulence factors

The DNase enzyme test was performed and gave results according to the method previously described by [15]. The Hemolysin test was also performed and the results were obtained according to the method previously described by [16].

Results and Discussion

Samples distribution

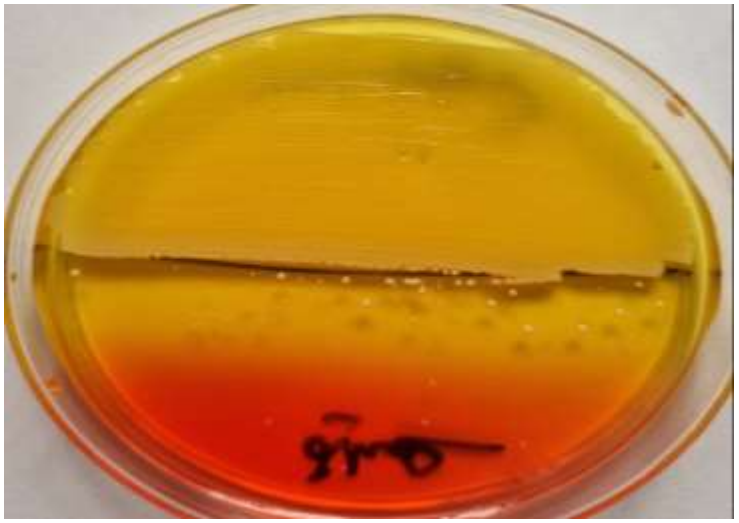
Samples were collected from patients' wounds as shown in Table (1). A total of 150 samples were taken from patients' wounds, with an average of 53(35.3%) samples for males and 97(64.7%) for females. The results found that all samples isolated from patients' wounds appeared positive for bacterial growth that were grown on optimal culture media such as blood agar and manitol salt agar. The results showed that 47 (31.3%) of the samples showed bacterial growth. These results are disagreeing with the study of the findings of Ekawati et al. [5], which indicated that the positive growth rate of Staphylococcus aureus isolated from wound samples reached (>99.9%). Staphylococcus aureus-related complications represent a significant clinical issue. The most frequent skin infections are caused by the pyogenic bacteria S. aureus and S. epidermidis.

Table (1): the distribution of samples depending on bacterial growth and gender

Gender	Male		Female		Total
Results	Number	%	Number	%	
Negative growth –ve	42	40.8	61	49.2	103 (68.7%)
Positive growth +ve	11	23.4	36	76.6	47 (31.3%)
Total	53	35.3	97	64.7	150 (100%)

Figure (1) illustrates the diameter and form of S. aureus isolates on Mannitol Salt agar. wherein the genus's bacterial isolates were identified using microscopic traits such the way Gram stain interacted with them. Furthermore, the color, texture, metallic luster, and pigment synthesis of the colony were all considered in determining the isolates and genera.

Figure (1): S. aureus on Mannitol Salt Agar



Then, biochemical tests were performed for Methicillin-resistant Staphylococcus aureus as shown in the table (2), and the diagnosis was confirmed using biochemical tests.

Table (2): S. aureus biochemical testing utilizing the biochemical approach

Isolate	Catalase	Oxidase	I	M	Vi	C	TSI		Urease	Motility	Mannitol Fermentation
			Indole	MR	VP	Citrate	TSI Reaction	H_2S Production			
S	+	-	-	+	-	-	K/K gas	+	+	-	+

Antibiotic Susceptibility Test

Polymorphous bacteria are one of the major and serious medical problems. This makes it difficult to choose the right treatment for the patient. Two methods were used for the purpose of testing the sensitivity of Methicillin-resistant Staphylococcus aureus to antibiotics, the method of diffusion on Muller-Hinton medium to determine the sensitivity of antibiotics against bacterial isolates as shown in the table (3).

Table (3): Antibiotic sensitivity against MRSA isolated from burns and wounds

Isolates	BEN	OX	CN	TOB	LEF	MOX	ERY	CL	VA	NIT	LIN	TRI
1	R	S	S	S	S	S	R	R	S	S	S	S
2	R	R	S	R	R	R	R	R	S	S	S	S
3	R	R	S	R	S	S	R	R	S	S	R	S
4	S	R	S	R	S	S	S	R	S	S	S	S
5	R	S	S	S	S	S	R	R	R	S	S	R
6	R	R	S	R	S	S	R	R	S	S	S	S
7	R	S	S	S	S	R	R	R	S	S	S	S
8	R	R	R	S	S	S	R	S	R	R	R	R
9	R	R	S	S	S	S	R	R	S	S	R	S
10	R	R	S	R	R	R	R	R	S	S	R	R
11	S	S	S	R	R	S	R	R	S	S	R	R
Resistance (%)	81.8	63.7	9.1	54.5	27.3	27.3	90.9	90.9	18.8	9.1	45.5	18.8

BEN: Benzylpenicillin, OX: Oxaccillin, CN: Gentamicin, TOB: Tobramycin, LEF: Levofloxacin, MOX: Moxifloxacin, ERY: Erythromycin, CL: Clindamycin, VA: Vancomycin, NIT: Nitrofurantion, TRI: Trimethoprim.

S. aureus carriage seems to be important for both the pathophysiology and epidemiology of infection. However, Numerous reports of its prevalence in healthy populations have been made; among them are those of S. aureus in adult nasal cavities in Iraq (43.2%), Turkish children (17.3%), Japanese adults (36%), and adult nasal cavities in the USA (32.4%) [17–19]. According to Chatterjee et al. [20], 52.3% of people had S. aureus nasal colonization overall. Onanuga and Temedie [21], however, demonstrated that out of 120 nares specimens that were examined, 33.3% of S. aureus isolates were found. However, Adesida et al. [22] found that medical students in Lagos, Nigeria had a much lower nasal colonization rate of 14.0%. These differences could be explained by the features of the population being studied. A population under antibiotic treatment at the time of sampling may have a significantly lower prevalence of S. aureus than a population from a hospital setting, where the high concentration of infectious patients may produce a

substantially higher frequency. Techniques used in culture and sampling may also contribute to variances. The S. aureus isolates used in this investigation seemed to have a high level of methicillin (Cefoxitin) resistance. Nonetheless, reports of MRSA isolates completely resistant to vancomycin (MIC ≥ 32 µg/ml) and bacteria with reduced resistance to the antibiotic (MIC, 8–16 µg/ml) date back to 1996 [23]. Additionally, our findings align with those of Brady et al. [24], who noted that every isolate exhibited resistance to penicillin and other β-lactam antibiotics. The current investigation, however, revealed a well-known development of vancomycin resistance, as evidenced by an increase in the rate of vancomycin-intermediate resistant S. aureus (VISA), which was 34 out of 106 (32.1%) in this study. Al-Geobory [25] in that 2.27% of people had vancomycin resistance. where 4 out of 50 (8%) samples of S. aureus include the VRSA isolate [26]. While Al-Hossainy [27] showed that the 20% VRSA among S. aureus. This implies that S. aureus nasal carriers are a significant risk factor for infection and the airborne spread of MRSA and VRSA within hospitals. Low levels of resistance revealed in this study to other antibiotics which is commonly used, these antibiotics include Erythromycin, Tetracycline, Gentamycin, Ciprofloxacin and Rifampin, the resistance percentages were 37.7%, 34.9%, 28.5%, 29.2% and 3.8%, respectively. Al-Geobory [25] demonstrated that the rates of resistant to Erythromycin, Tetracycline, Gentamycin, Ciprofloxacin and Rifampin were 34.09%, 31.81, 20.45%, 13.63% and 13.63%.

Virulence factors

DNase enzyme test

A DNase test was conducted for clinical isolates of S. aureus, which were isolated from burns and wounds, and the results were 100% in terms of the susceptibility of S. aureus isolates to DNase results, as shown in Figure (4 and 5).

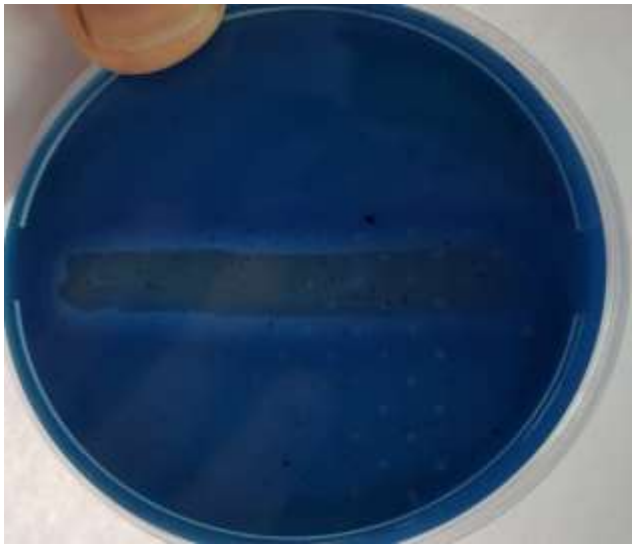


Figure (4): DNase enzyme test

Figure (5): DNase enzyme test

The results also showed that the percentage of DNase enzyme results for S. aureus isolates reached 100%. The results of the current study agreed with Abdullah [28] study, which indicated the ability of Staphylococcus aureus to the results of some extracellular enzymes as virulence factors, where the isolates of Staphylococcus aureus were 100% productive of DNase. The results

of the current study also agreed with Lagace-Wiens et al [29]. Which indicated the ability of staphylococci to the results of the extracellular DNase enzyme as a virulence factor, as it was 100% for the DNase enzyme, and according to the study, the DNase test has the lowest amount of false positive isolates and is the most specific of all the tests.

Hemolysin

We cultured 47 S. aureus isolates on blood agar medium to determine their hemolysin production capacity. Findings revealed variations in the proportions of S. aureus isolates to hemolysin production; 39 (82.98%) of the isolates exhibited complete hemolysis, as indicated by a semi-transparent appearance surrounding a colony, indicating the isolates' capacity to make hemolysin, as in figure (6).

Figure (6): The rate of isolates positive for hemolysin.

The results of the current study agreed with Al-Jundiy [30], which stated that about 85.1% of S. aureus isolates were hemolytic. The origin of the blood cells that are used in the culture media influences the test procedure and is dictated by the test method's capacity to identify β-hemolysis, and the presence of serum and cholesterol in the blood used, which inhibits the process of hemolysis, are the most significant factors that affect a bacteria's ability to produce hemolysis [31]. Therefore, S. aureus has the ability to produce hemolysin due to the osmotic properties it possesses for hemolysis or due to the formation of porous or cytotoxic holes in the cell to dissolve types of human blood cells [32].

Conclusion

It is concluded from the current study that S. aureus that was isolated from wounds was highly resistant to antibiotics and had dangerous harmful factors that increased the bacterial level in infections.-spasi-

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